Implementation of Isotope-labeled Internal Standards in LC-MS based Global Metabolomics

Student: Ida Havikbotn
Supervisors: Katja Benedikte Prestø Elgstøen and Eirik Sundby

BACKGROUND

Metabolomics is the study of metabolites where the aim is to identify and quantify all metabolites in a biological sample [1]. It is a fast-growing field within biomedical research and has the potential to revolutionize clinical diagnostics. Global metabolomics is a powerful tool within this field which can measure several thousand compounds simultaneously. Oslo University Hospital has developed and implemented a method within such global metabolomics for a variety of biological samples, and it is used in clinical diagnosis as well as scientific research. However, there are disadvantages to the current approach, including the quality assurance of data and test answers, and the inability to quantify globally.

AIM OF STUDY

The objective of this study is to assess carefully selected isotope-labelled internal standards (ILIS) in order to improve the quality assurance and enable metabolite quantification. Another question being emphasized is how the ILIS affects the endogenous metabolites on a global scale.

Isotope-labelled Internal Standards

- Compounds where several atoms within a molecule is replaced by their stable, non-radioactive isotopes (e.g. deuterium (^{2}H or D), ^{13}C or ^{15}N) [2]
- When labelling a standard with an isotope the mass increases , making the internal standard more ideal for analysis using MS

Deuterium
It is found naturally in hydrogen compounds (0,0156 %) and is an isotope where the nucleus has both a proton and a neutron [3].
Weight natural hydrogen: 1,008 g/mol
Weight deuterium: 2,014 g/mol

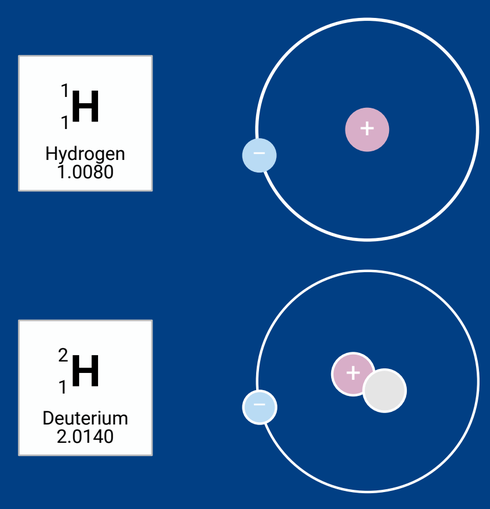

Figure 1: Hydrogen and deuterium.

UHPLC-MS

- Apparatus consisting of a Dionex Ulltimate 3000 UHPLC connected to a Q Exactive Orbitrap ESI- MS from Thermo Fisher Scientific
- Separates compounds in advance of detection
- System settings have been optimized to provide optimum separation and detection of the greatest number of compounds
- The mass spectroscopy is the detector in this system

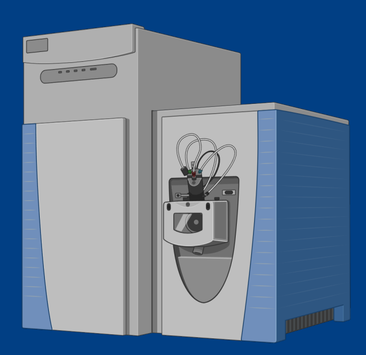

Figure 2: UHPLC-MS instrument.

METHOD

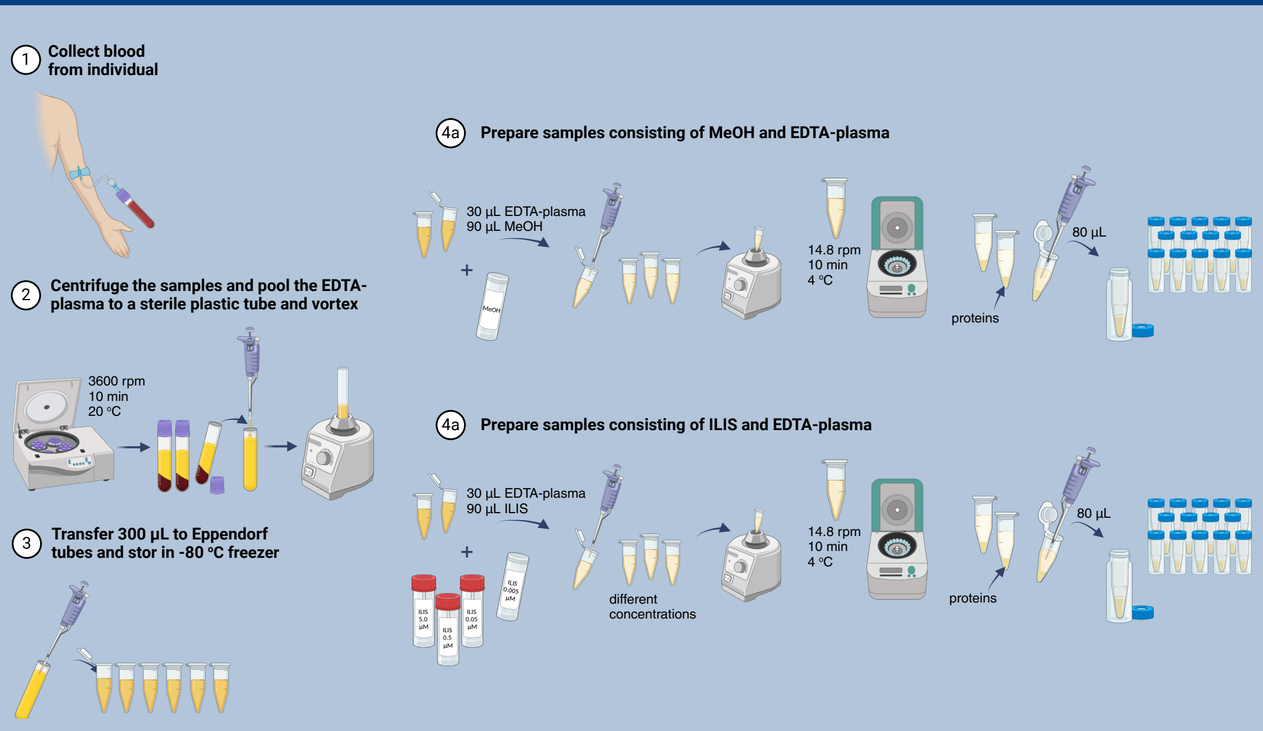

Figure 3: Sample preparation using both MeOH and ILIS before further analysis on the UHPLC-MS instrument.

Preparation of isotope-labelled internal standard:
Four isotope-labelled compounds (Alanine-d4, Tryptophan-d5, C-18-Acylcarnitine, Uracil-15N2) were diluted with MeOH. EquiSPLASH (isotope-labelled lipids) was added, resulting in a final concentration of approximately 5.0 µM.

Sample preparation:
Blood was collected and centrifuged in order to extract the EDTA-plasma. The prepared samples was consisting of 30 µL EDTA-plasma and ether 90 mL MeOH or ILIS. After centrifugation in order to precipitate proteins, 80 µL was transferred to vials for further analysis on UHPLC-MS. The sample preparation is illustrated in Figure 3.

RESULTS AND FUTURE WORK

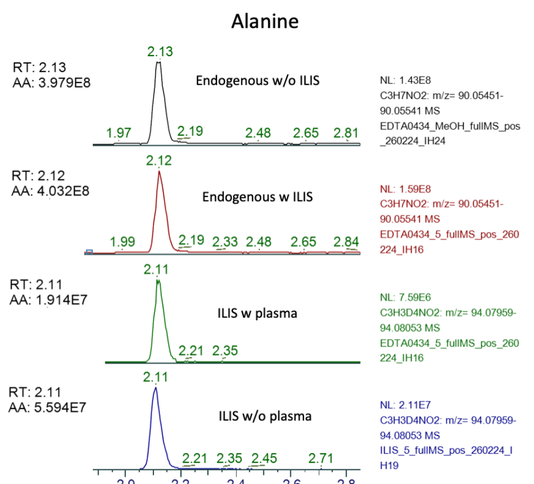

Figure 4: How does Alanine d4 affect the EDTA-sample?

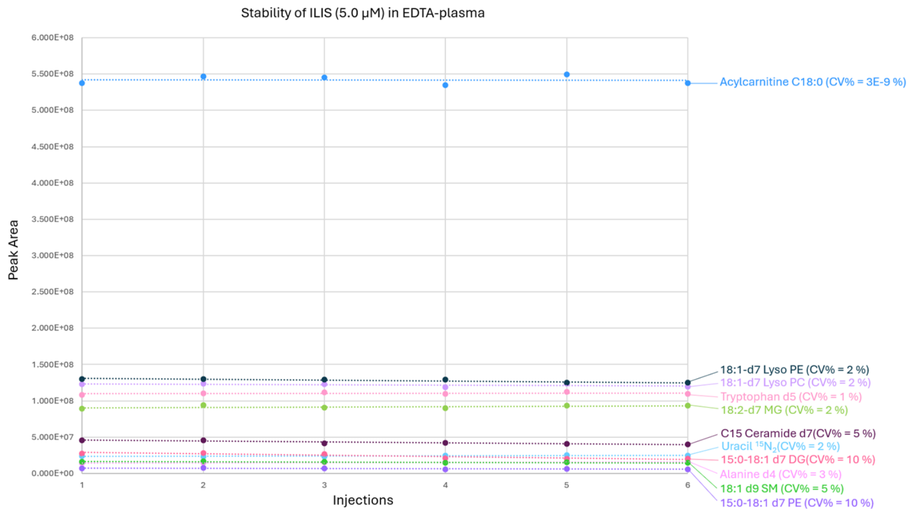

Figure 5: Will the ILIS stay stable in biological samples?

REFERENCES

1. Beger RD, Dunn W, Schmidt MA, Gross SS, Kirwan JA, Cascante M, et al. Metabolomics enables precision medicine: "A White Paper, Community Perspective". Official journal of the Metabolomic Society. 2016;12(10):149.
2. Tobin J. Designing Stable Isotope Labeled Internal Standards [Internet]. Mississauga: Acanthus research; 2022 [updated 2022 Jan 11; cited 2024 24 Jan]. Available from: https://acanthusresearch.com/generalblog/designing-stable-isotope-labeled-internal-standards/
3. Encyclopaedia TEo. deuterium [Internet]. Britannica; 2023 [updated 2023 Nov 28; cited 2024 23 Jan]. Available from: https://www.britannica.com/science/deuterium

Norwegian University of Science and Technology NTNU

Oslo University Hospital